



UNIVERSITI PUTRA MALAYSIA

**REPRODUCTIVE BIOLOGY OF HORSESHOE CRAB
(CARCINOSCORPLUS ROTUNDICAUDE) WITH EMPHASIS ON
GONAD AND GAMETE MORPHOLOGY**

PARVANEH HAJEB.

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REPRODUCTIVE BIOLOGY OF HORSESHOE CRAB (*Carcinoscorpius rotundicauda*) WITH EMPHASIS ON GONAD AND GAMETE MORPHOLOGY

By

PARVANEH HAJEB

Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in
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October 2005



Dedicated

To my dearly beloved family for all their love, supports, understanding and patient.

Abstract of thesis presented to the Senate of Universiti Putra Malaysia in
fulfilment of the requirements for the degree of Master of Science

**Reproductive Biology of Horseshoe Crab (*CARCINOSCORPIUS ROTUNDICAUDA*)
With Emphasis on Gonad and Gamete Morphology**

By

PARVANEH HAJEB

October 2005

Chairman : Annie Christianus, PhD

Faculty : Agriculture

Samples of adult *Carcinoscorpius rotundicauda* caught from Pulau Lumut, Selangor, from November 2003 to February 2005 were conditioned in the Hatchery Unit, Universiti Putra Malaysia and used for all the experiments.

C. rotundicauda was identified based on its morphological characteristics and at the same time age determination was carried out. It is the smallest of four extant species of horseshoe crabs. Male adults were smaller than females. Based on the size estimation, it was found that 75% of collected *C. rotundicauda* males were of 10 to 11 years old (after 14th molt), while 59% of the females were 11 to 12 years old (after 15th molt).

The male reproductive organ was investigated morphologically and histologically. It consisted of testis and spermiducts network which was filled with spermatogenic cells (spermatogonia, primary and secondary spermatocytes) that lied along the basement membrane with spermatozoa close to the testicular lumen. SEM observations showed that a mature

spermatozoa consisted of a head with a cap-like acrosomal vesicle, midpiece and a long flagellum. The average size of the sperm head was 4.6 μm while the flagellum was 33 μm in length. The acrosomal filament formed 11 helical coils around the nucleus. Observation on the cross-section of the flagellum showed a 9+0 axoneme pattern without the central tubules.

Morphology and histology of mature ovary and egg were studied. The female gonad was located in the prosomal part, consisted of ovarian tubes and oviducts which opened as a pair of gonopores on the ventral side of the opisthosoma. A *C. rotundicauda* egg (of 2.25 to 2.58 mm in diameter) had a large yolk covered with an elastic chorion with two layers, the outer basement lamina (3.03 μm thickness) and the inner vitelline envelope (43.6 μm thickness). Fecundity of *C. rotundicauda* ranged from 993 to 7937 eggs per adult, increasing with body size and weight.

Interactions between egg and sperm were observed under SEM. Spermatozoa were attached to the outer layer of the egg's chorion. In this study, high sperm concentration ($23 - 30 \times 10^6$ sperm/ml) resulted in numerous sperms attached to the egg surface. Even though many sperms can attach to the egg surface and pass through the egg's envelope, only one will transfer its nucleus into the vitellus and fertilize the egg.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia
sebagai memenuhi keperluan untuk ijazah Master Sains

**Biologi Pembiakan Belangkas (*CARCINOSCORPIUS ROTUNDICAUDA*)
dengan Penumpuan ke atas Morfologi Gonad dan Gamet**

Oleh

PARVANEH HAJEB

Oktober 2005

Pengerusi : Annie Christianus, PhD

Fakulti : Pertanian

Sampel *Carcinoscorpius rotundicauda* dewasa didapati dari Pulau Lumut, Selangor, dari November 2003 hingga Februari 2005 disesuaikan di Unit Penetasan, Universiti Putra Malaysia dan digunakan untuk semua eksperimen.

C. rotundicauda dikenalpasti berdasarkan ciri-ciri morfologinya dan pada masa yang sama penentuan umur turut dijalankan. Ia adalah spesies yang terkecil di antara empat spesies yang masih hidup. Belangkas jantan dewasa adalah kecil berbanding dengan betina. Berdasarkan anggaran saiz, didapati bahawa 75% belangkas jantan yang dikutip adalah berada pada lingkungan umur 10 hingga 11 tahun (selepas 14 kali bersalin kulit), manakala 59% dari betina berumur 11 hingga 12 tahun (selepas 15 kali bersalin kulit).

Organ pembiakan belangkas jantan telah dikaji dari segi morfologi dan histologi. Ia terdiri dari testis dan jaringan duktus sperma yang dipenuhi oleh sel-sel spermatogenik (spermatogonia, spermatosit primer dan sekunder)

yang terletak di sepanjang membran dasar dengan spermatozoa terdapat hampir ke lumen testikular. Pemerhatian SEM menunjukkan bahawa spermatozoa matang mempunyai satu kepala dengan vesikel akrosom menyerupai topi, bahagian tengah dan satu flagellum yang panjang. Saiz purata kepala sperma adalah $4.6\text{ }\mu\text{m}$ manakala flagellum $33\text{ }\mu\text{m}$ panjang. Filamen akrosom membentuk 11 lingkaran helik mengelilingi nukleus. Pemerhatian ke atas keratan rentas flagellum menunjukkan corak aksonema 9+0 tanpa tubul tengah.

Morfologi dan histologi ovari dan telur matang telah dikaji. Gonad betina terletak di bahagian prosoma, terdiri dari tiub ovari, duktus ovari yang terbuka sebagai sepasang gonopor pada ventral opistosoma. Telur *C. rotundicauda* (berdiameter 2.25 hingga 2.58 mm) mempunyai satu yolka yang besar diselaputi korion yang elastik dan ianya mempunyai dua lapisan iaitu lamina dasar pada bahagian luar ($3.03\text{ }\mu\text{m}$ tebal) dan selaput vitelin ($43.6\text{ }\mu\text{m}$ tebal) pada bahagian dalam. Fekunditi *C. rotundicauda* adalah pada julat 993 hingga 7937 telur untuk seekor dewasa yang meningkat dengan peningkatan saiz dan berat badan.

Interaksi di antara telur dan sperma diperhatikan melalui SEM. Spermatozoa melekat di permukaan luar korion telur. Dalam kajian ini, kepekatan sperma yang tinggi ($23 - 30 \times 10^6$ sperm/ml) menyebabkan banyak sperma melekat pada permukaan telur. Walaupun banyak sperma boleh melekat pada permukaan telur dan menembusi selaput telur, hanya satu sahaja yang akan memindahkan nukleusnya ke dalam vitellus dan mensenyawakan telur.

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I certify that an Examination Committee met on 18th October 2005 to conduct the final examination of Parvaneh Hajeb on her Master of Science thesis entitled "Reproductive Biology of Horseshoe Crab (*Carcinoscorpius rotundicauda*) with Emphasis on Gonad and Gamete Morphology" in accordance with Universiti Pertanian Malaysia (Higher Degree) Act 1980 and Universiti Pertanian Malaysia (Higher Degree) Regulations 1981. The Committee recommends that the candidate be awarded the relevant degree. Members of the Examination Committee are as follows:

Mohd. Salleh Kamarudin, PhD

Associate Professor
Faculty of Agriculture
Universiti Putra Malaysia
(Chairman)

Siti Khalijah Daud, PhD

Associate Professor
Faculty of Science
Universiti Putra Malaysia
(Internal Examiner)

Mustafa Kamal Abdul Satar, PhD

Lecturer
Faculty of Agriculture
Universiti Putra Malaysia
(Internal Examiner)

Chong Ving Ching, PhD

Professor
Institute of Biological Science
Universiti Malaya
(External Examiner)



ZAKARIAH ABD RASHID, PhD

Professor/Deputy Dean
School of Graduate Studies
Universiti Putra Malaysia

Date: **27 DEC 2005**



This thesis submitted to the Senate of Universiti Putra Malaysia has been accepted as fulfilment of the requirement for the degree of Master of Science. The members of the Supervisory Committee are as follows:

Annie Christianus, PhD

Lecturer
Faculty of Agriculture
Universiti Putra Malaysia
(Chairman)

Che Roos Saad, PhD

Associate Professor
Faculty of Agriculture
Universiti Putra Malaysia
(Member)

Aziz Arshad, PhD

Associate Professor
Faculty of Science
Universiti Putra Malaysia
(Member)



AINI IDERIS, PhD
Professor / Dean
School of Graduate Studies
Universiti Putra Malaysia

Date: 12 JAN 2006

DECLARATION

I hereby declare that this thesis is based on my original work except for quotations and citations which have been duly acknowledged. I also declare that it has not been previously or concurrently submitted for any other degree at UPM or other institutions.

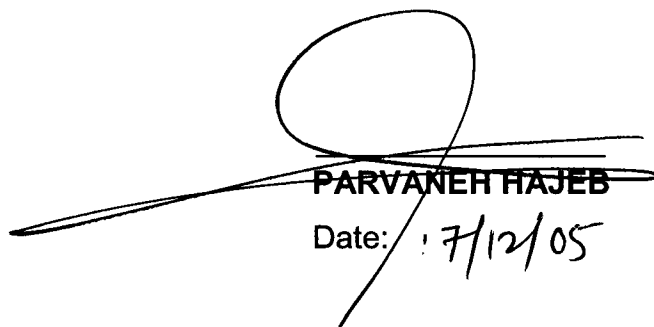

PARVANEH HAJEB
Date: 7/12/05

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LIST OF ABBREVIATIONS

a	Acrosome
Aa	Anal angle
Asp	Anterior spermiduct
Bm	Basement membrane
Bma	Branchio-thoracic muscle attachment
Ce	Compound eyes
Ch	Chelicerae
cm	Centimetre
CR	Cardiac region
EcR	Extracardiac region
Ema	Entro-branchial muscle attachment
Ew	Distance between the two compound eyes
F	Flagellum
g	gram
Gn	Gonopores
GSI	Gonadosomatic index
H	Sperm head
H & E	Haematoxylin and Eosin
In	Intestine
LF	Longitudinal furrow
LSp	Lateral spermiduct
M	Midpiece
Ma	Marginal area
Me	Median eyes

MI	Median line
ml	millilitre
mm	millimetre
µm	micrometer
Mr	Median ridge
Ms	Marginal spine
MSp	Main spermiducts
n	Sperm nucleus
OI	Length of the opisthosoma
On	Opisthosomatic notch
Op	Opercular pleurites
Or	Ophthalmic ridge
Ow	Maximum width of the opisthosoma
PI	Length of the prosoma
Plr	Postero-lateral ridge
Pr	Posterior ridge
Psp	Primary spermatocyte
Pw	Maximum width of the prosoma
S1-S6	Length of the first to sixth opisthosomal spine
SEM	Scanning electron microscope
Spg	Spermatogonia
Spz	Spermatozoa
SR	Subophthalmic region
Ssp	Secondary spermatocytes
TEM	Transmission electron microscope
TI	Length of the telson

Tn	Testicular network
Tt	Testicular tube
Tw	Width of the telson
Vms	Ventro-mesal spine
Vsa	Ventro-subfrontal



CHAPTER 1

INTRODUCTION

1.1 Background of study

The horseshoe crab is one of the Earth's oldest creatures, appearing over hundred million of years before the dinosaurs. The horseshoe crab is not a crab. Instead it is closely related to spider and scorpions. According to the fossil records, the horseshoe crab's appearance has remained unchanged for more than 300 million years. Therefore they are often called the "living fossils" (Iwanaga and Kawabata, 1998). Despite surviving for 300 million years and successfully passing through several mass extinctions, the horseshoe crab is not exempted from disappearing from this earth. The modern horseshoe crab has evolved little since their fossilized ancestor roamed the ocean floor (Fox, 2001).

There are only four living species of horseshoe crabs worldwide. In the Atlantic coast is *Limulus polyphemus* (Linnaeus, 1819). In Indo-Pacific waters is the Mangrove horseshoe crab, *Carcinoscorpius rotundicauda* (Laterille, 1802), the smallest among all the existing horseshoe crabs, and the Coastal horseshoe crab, *Tachypleus gigas* (Muller, 1758). In the waters of Japan, China and southern Sabah is the Chinese horseshoe crab *Tachypleus tridentatus* (Leach, 1819). These species can be differentiated by their size, colour and shape of tail. Two of these species (*T. gigas* and *C. rotundicauda*) can be found in Singapore (Sekiguchi, 1988). The Coastal horseshoe crab (*Tachypleus gigas*) is the largest

(diameter up to 25 cm). It is greyish in colour with a triangular and serrated telson. The Mangrove horseshoe crab (*Carcinoscorpius rotundicauda*) is the smallest (diameter up to 15 cm), brownish with a rounded telson (Sekiguchi, 1988). These species of horseshoe crabs belong to the subclass Xiphosura, are the only surviving members of the Merostomata. Comparison of this amazing animal with some fossil remains found in Silurian deposits shows that they have not changed much in nearly 400 million years (Kozloff, 1990). Today's horseshoe crabs closely resemble those known from Silurian period (Fox, 2001).

This invertebrate gets its name from the large, rounded, horseshoe-shaped carapace or shell, which covers its legs and softer body parts. The name "horseshoe crab" is partially an accurate correct description of the animal. Its upper body the prosomatic carapace has a horseshoe shape. It has book-shaped gills but different from the crabs with no jaws and antenna (Riska, 1981).

1.2 The importance of the study

The horseshoe crabs lived at the time of dinosaurs. Yet a sharp decline in its present population brings fear that it may share the same fate as other prehistoric species.

Horseshoe crabs species are important ecologically; fishermen use them as bait and a product derived from one species of the crab's blood are used by pharmaceutical companies to produce Limulus Amebocyte Lysate (LAL), a